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Immunology

Interleukin 1 participates in the development of anti-*Listeria* responses in normal and SCID mice

(monoclonal antibody/natural immunity/cytokine/microbial infection)

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ABSTRACT **Using T- and B-cell deficient C.B-17 mice with the *scid*[3] mutation, we have previously documented the existence of a T-cell-independent but interferon γ-dependent pathway of macrophage activation that confers upon the host partial resistance to the facultative intracellular bacterium *Listeria monocytogenes*. This pathway is operative in both normal and SCID mice and consists of at least four components: interferon γ, tumor necrosis factor, macrophages, and natural killer cells. Here we demonstrate that interleukin 1 also participates in this pathway but at a different site of action. Using monoclonal antibodies that neutralize the biologic activities of interleukin 1α and interleukin 1β, we document that interleukin 1 participates neither directly in the induction of interferon γ from isolated SCID natural killer cells nor in the antigen-specific activation of $CD4^+$ T cells derived from *Listeria*-immune C.B-17 mice. In contrast, injection of a mixture of anti-interleukin 1α, anti-interleukin 1β, and a newly derived monoclonal antibody specific for the murine type I interleukin-1 receptor into either SCID or normal C.B-17 mice blocked the *in vivo* elaboration of class II major histocompatibility complex-positive macrophages after infection of the animals with *Listeria*. Moreover, SCID mice treated with the anti-interleukin-1 mixture failed to control the growth of *Listeria in vivo* and eventually succumbed to the infection. These results document that endogenously produced interleukin 1 plays an obligate role in the *Listeria*-dependent induction of activated macrophages *in vivo* and demonstrate that the action of interleukin 1 is distinct from the generation of natural killer cell-derived interferon γ.**

During the past few years, several laboratories have begun to assess the role of cytokines in promoting immune responses to microbial pathogens and protein antigens. We have approached this subject in our laboratories by using the well-characterized model of *Listeria monocytogenes* infection in mice. This work has led to the recognition that murine resistance to infection by this intracellular facultative bacterium depends on T-cell-independent as well as T-cell-dependent mechanisms of macrophage activation. Using neutralizing cytokine-specific monoclonal antibodies (mAbs) in experiments *in vivo*, we showed that interferon γ (IFN-γ) and tumor necrosis factor (TNF) played obligate roles in promoting resistance to *Listeria* in either normal mice or lymphocyte-deficient SCID (*scid*[3]) mice (1, 2). Mice treated with IFN-γ- or TNF-specific mAbs failed to elaborate class II major histocompatibility complex (MHC)-positive macrophages after infection with a sublethal dose of *Listeria*, were unable to control the replication of the bacteria in the liver and spleen, and eventually succumbed to the infection. A similar effect was observed in SCID mice treated with antibodies to natural killer (NK) cells. These studies suggested that NK-cell-derived IFN-γ played an important physiologic role in promoting innate resistance to bacteria and indicated that TNF also participated in this process. More detailed information was obtained in subsequent *in vitro* experiments (2, 3). Addition of live or dead *Listeria* to cultures of SCID spleen cells induced the rapid appearance of TNF in culture fluids, followed by the elaboration of IFN-γ. Similar results were obtained when purified populations of macrophages and NK cells were used. The latter were derived by culture of SCID spleen cells in interleukin 2 (IL-2). Addition of anti-TNF to the *Listeria*-pulsed SCID cell culture inhibited IFN-γ production, whereas addition of anti-IFN-γ to the culture did not inhibit generation of TNF. Finally, highly purified recombinant murine TNF in combination with bacterial products induced IFN-γ production by cultures of NK cells propagated in the presence of IL-2.

On the basis of these experiments, we proposed the following model for the development of *Listeria* resistance in mice (4). Shortly after infection, macrophages in the naive host ingest, kill, and digest live *Listeria* and release TNF and an as-yet-undefined second product (possibly a processed microbial product). These two agents then stimulate NK cells to produce IFN-γ. NK-cell-derived IFN-γ in turn activates macrophages to express class II MHC antigens and enhanced antimicrobial activity. The latter facilitates the destruction of *Listeria* residing within activated macrophages and thus hinders the spread of the *Listeria* infection *in vivo*. In the SCID mouse, the absence of T-cell immunity permits bacteria residing in other cell types to escape destruction, and therefore these hosts become chronically infected with *Listeria*. In normal mice, the destruction and processing of *Listeria* by activated macrophages facilitates the presentation of *Listeria* antigen to T cells and thereby promotes the induction of *Listeria*-specific $CD4^+$ and $CD8^+$ T cells that eventually effect sterilizing immunity. Thus we believe that the T-cell-independent pathway of macrophage activation observed in the SCID mouse may represent the initiating events of (at least some types of) antimicrobial responses.

In the current communication we now extend our analyses to examine the role of IL-1 in the development of anti-*Listeria* responses in normal and SCID mice. Using preexisting neutralizing mAbs specific for murine IL-1α and -β and newly derived blocking hamster mAbs specific for the type I IL-1 receptor, we demonstrate that whereas endogenously pro-

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Abbreviations: mAb, monoclonal antibody; IFN-γ, interferon γ; TNF, tumor necrosis factor; SCID, severe combined immunodeficiency syndrome; MHC, major histocompatibility complex; NK, natural killer; IL, interleukin; HKLM, heat-killed *Listeria monocytogenes*; PEM, peritoneal exudate macrophages.

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duced IL-1 participates *in vitro* in neither the antigen-dependent activation of *Listeria*-immune T cells nor the TNF-dependent activation of NK cells to produce IFN-γ, it is required *in vivo* for the induction of class II MHC-positive macrophages in *Listeria*-infected normal and SCID mice.

MATERIALS AND METHODS

C.B-17 and SCID Mice. Male and female C.B-17 and SCID (5) mice were bred at Washington University and were used between the ages of 9 and 13 weeks.

mAbs. The following previously described and characterized mAbs were used: L2-3D9 is an irrelevant hamster mAb that has no known reactivity with any recognized natural murine cell or protein (6). TN3-19.12 is a TNF-specific hamster mAb that neutralizes murine and rat TNF-α and probably also TNF-β (6). ALF-161 (7) and B122 (8) are neutralizing hamster mAbs specific for IL-1α and IL-1β, respectively, and do not crossreact with the other IL-1 form. H22 is an IFN-γ-specific neutralizing hamster mAb (9). MP-20F3 is a rat IgG1 mAb that neutralizes murine IL-6 (10) and was a generous gift from Marvin Siegel (Schering, Bloomfield, NJ) and John Abrams (DNAX, Palo Alto, CA). M15 (11) and 35F5 (12) are rat mAbs specific for the murine type I IL-1 receptor that block binding of IL-1 to receptor-bearing cells. All mAbs were purified to homogeneity by ion-exchange or staphylococcal protein A chromatography and contained less than 50 endotoxin units/mg of protein as determined by using the QCL-1000 endotoxin quantitation kit (Whittaker Bioproducts).

Generation of Blocking Hamster mAbs Specific for the Murine Type I IL-1 Receptor. Type I IL-1 receptor-specific mAbs were produced by using previously described methods (9). An Armenian hamster was immunized with highly purified preparations of the murine type I IL-1 receptor extracellular domain (13), and splenocytes from the immune hamster were fused to the P3X63.Ag8.653 murine myeloma cell line by using polyethylene glycol. Supernatants from hypoxanthine/aminopterin/thymidine (HAT)-resistant cultures were screened for the ability to block the binding of 1 ng of ^{125}I-labeled human IL-1α to 2.5×10^6 murine EL-4 cells. This fusion resulted in 163/348 growth positive/total wells. Four cultures (JAMA-83, JAMA-84, JAMA-110, and JAMA-147) blocked binding. The four hybridomas were cloned three times by limiting dilution and mAbs were purified from spent culture supernatants by affinity HPLC on protein A-Affi-Prep (Bio-Rad).

Radioligand or Antibody Binding Assays. Purified human IL-1α and purified JAMA mAb were radiolabeled with ^{125}I to specific activities of 3–15 μCi (1 Ci = 37 GBq)/μg by using the chloramine T method as described (14). Radioligand binding analysis was conducted essentially as described elsewhere (15), using 2.5×10^6 EL-4 cells in a total volume of 100 μl. Cell-associated and free radioactivity were separated by centrifugation through a 6:4 (vol/vol) mixture of dibutyl phthalate/dioctyl phthalate oil. Binding data were analyzed by the method of Scatchard (16). The EL-4 line used in our laboratory expressed 2300 type I IL-1 receptors per cell and displayed a single K_a for ligand of 2.8×10^9 M^{-1} at 4°C.

***In Vitro* Biologic Assays.** CBA/J mice, age 8–12 weeks (The Jackson Laboratory) were injected i.p. with 2×10^4 live *Listeria* (0.1 LD$_{50}$). Six days later the spleens were harvested and CD4$^+$ T cells were purified through columns of nylon wool followed by treatment with anti-CD8 and complement as previously described (17). The resulting cells were then restimulated *in vitro* with 10^6–10^7 heat-killed *Listeria monocytogenes* (HKLM) per ml in RPMI 1640 medium supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin (50 units/ml), streptomycin (50 μg/ml), 2-mercaptoethanol (50 μM), and indomethacin (5 μg/ml) in a volume of 200 μl in 96-well flat-bottom plates. T-cell proliferation was measured by incorporation of [^{3}H]thymidine (0.4 μCi per well) during the final 18 hr of a 72-hr culture using 2×10^5 CD4$^+$ spleen cells per well. To measure cytokine production, cultures of 6×10^5 cells per well were established and supernatants were harvested after 24 or 48 hr and assayed for IL-2 or IFN-γ, respectively. IL-2 was quantitated by bioassay using the CTLL line (18) and IFN-γ by ELISA as described previously (1). mAbs to IL-1α and IL-1β were used as a mixture at 100 μg/ml each and anti-IL-6 at 10 μg/ml. The mAbs GK1.5 (anti-CD4) and 6.72 (anti-CD8) were prepared as saturated ammonium sulfate precipitates at concentrations of 33.2 and 28.4 mg/ml, respectively.

The ability of IL-1 to stimulate proliferation of the D10.G4 murine T-cell clone in the presence of Con A was determined as previously described (19).

Analysis of the Effects of Anti-IL-1 mAbs *in Vivo*. The basic experimental protocol consisted of injecting mice i.p. on day −2 with 600 μg of purified mAb (or antibody mixture) diluted in pyrogen-free saline and then infecting the mice i.p. with 5×10^3 *Listeria monocytogenes* on day 0. In the case of the anti-IL-1 antibodies, the mice received either a pool of mAb that contained 200 μg each of anti-IL-1α (ALF-161), anti-IL-1β (B122), and anti-type I IL-1 receptor (JAMA-147) or various amounts of the individual mAb. Three to 4 days later the mice were sacrificed and peritoneal exudate macrophages (PEM) and spleens were collected as described (20, 21). Analysis of class II MHC expression on PEM was conducted on 5×10^5 PEM that had adhered to coverslips by culture for 2 hr at 37°C. The adherent cells were fixed in 1% paraformaldehyde for 10 min at 4°C, washed, and then examined for I-A^d expression by immunofluorescence as described (21), using the MKD6 anti-I-A^d monoclonal antibody as the first layer and fluorescein-conjugated F(ab')$_2$ rabbit anti-mouse IgG as the second layer. The percentage of I-A^d-positive macrophages in untreated C.B-17 or SCID mice ranged from 5% to 20%. The number of viable bacteria in the spleens of infected animals was quantitated as described (20).

RESULTS AND DISCUSSION

Endogenously Produced IL-1 Does Not Activate NK Cells to Produce IFN-γ or Promote Antigen-Specific T-Cell Activation. Our previously reported *in vitro* experiments failed to reveal a role for IL-1 in the generation of IFN-γ from NK cells (2, 3, 20). Whereas anti-TNF- and anti-IFN-γ-specific mAbs ablated the ability of SCID spleen cells to produce IFN-γ when cultured with HKLM, no inhibition was observed when IL-1α/β-specific antibodies were added to the cultures. Moreover, addition of purified IL-1 to the SCID cell cultures did not enhance the production of IFN-γ. Here we examined whether addition of neutralizing IL-1α- or β-specific mAb to cultures of spleen cells derived from *Listeria*-immune mice could inhibit T-cell responses induced by stimulation of the cultures with antigen (HKLM). Fig. 1 demonstrates that IL-1-specific antibodies were unable to inhibit either T-cell proliferative responses to HKLM (Fig. 1*A*) or the production of either IL-2 (Fig. 1*B*) or IFN-γ (Fig. 1*C*). The CD4$^+$ T-cell response to HKLM was also not inhibited by anti-IL-6 or anti-TNF mAb, nor was the response altered by a mixture of mAbs specific for IL-1α, IL-1β, IL-6, and TNF (data not shown). The same anti-cytokine mixtures also failed to inhibit IFN-γ production by *Listeria*-specific CD4$^+$ T-cell clones (data not shown). Thus, in this model, IL-1 does not appear to be important either in the direct activation of NK cells leading to IFN-γ production or in the antigen-specific activation of *Listeria*-specific T cells.

Characterization of IL-1 Receptor-Specific mAbs. We next wished to examine whether IL-1 played a role *in vivo* in mediating anti-*Listeria* responses. To ensure that we could

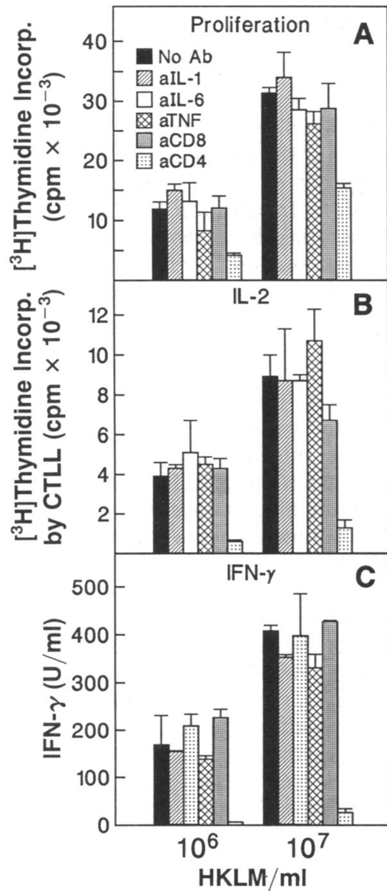

FIG. 1. mAbs specific for murine cytokines do not inhibit $CD4^+$ T-cell responses to HKLM. $CD4^+$ spleen cells were purified from CBA/J mice infected 6 days previously with 2×10^4 live *Listeria*. Two-hundred-microliter cultures were established containing $2–6 \times 10^5$ spleen cells, 2–20 μg of different purified mAb (key in *A*; a- indicates anti-), and two concentrations of HKLM. Spleen cell proliferation (*A*) and production of IL-2 (*B*) and IFN-γ (*C*) were determined. The $CD4^+$ nature of the spleen cell response was confirmed by inhibition with anti-CD4 but not anti-CD8 mAb. Data are expressed as the mean ± SD from one of two similar experiments.

completely neutralize all IL-1 effects *in vivo*, we used a combination of anti-IL-1 and anti-IL-1 receptor reagents. However, the two currently available rat mAbs that are specific for the murine type I IL-1 receptor [M15 (11) and 35F5 (12)] are only marginally effective *in vivo* because of their relatively weak ability to block IL-1-dependent cellular responses. Therefore, we decided to generate our own mAbs in hamsters, since we have used this approach successfully in the past to produce unique high-affinity mAb reagents to a variety of other murine cytokines and cytokine receptors. When the purified extracellular domain of the murine type I IL-1 receptor was used as antigen (13), four distinct mAbs were produced and identified by their ability to block binding of ^{125}I-labeled human IL-1α to the murine EL-4 thymoma cell line. All four mAbs reacted with the either T cells or fibroblasts that specifically expressed the type I IL-1 receptor as determined by (*i*) inhibition of radioligand binding, (*ii*) direct binding analysis using labeled antibody, and (*iii*) flow cytometry using biotinylated mAb and a streptavidin-phycoerythrin conjugate. None of the mAbs reacted with the murine pre-B cell line 70Z/3, which expresses only the type II IL-1 receptor. Fig. 2 compares the abilities of the four mAbs to inhibit binding of 1 ng of radiolabeled IL-1 to 2.5×10^6 EL-4. All four hamster antibodies blocked binding of ^{125}I-labeled IL-1 in a dose-dependent manner, although with

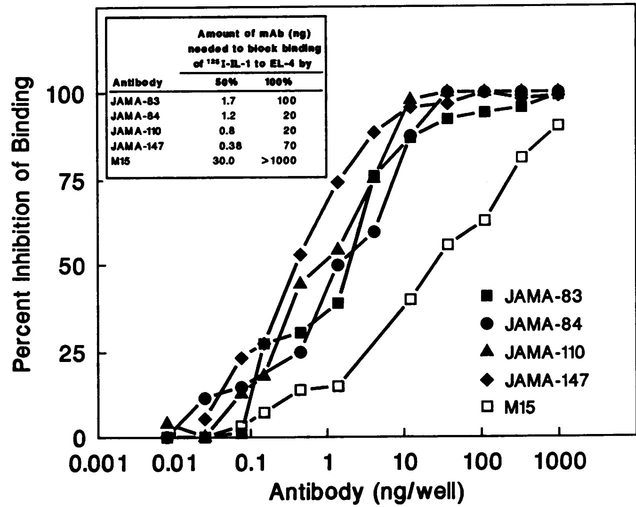

FIG. 2. Comparison of the ability of different JAMA mAbs to block binding of ^{125}I-labeled human IL-1α to EL-4 cells. Different amounts of purified JAMA-83 (■), JAMA-84 (●), JAMA-110 (▲), JAMA-147 (◆), or M15 (□) mAbs were incubated with 2.5×10^6 EL-4 in a total volume of 75 μl for 60 min at 4°C, and then 25 μl containing 1 ng of ^{125}I-labeled IL-1α was added and the incubation was continued for another 30 min. Seventy-five microliters of the suspension was layered onto a 240-μl cushion of dibutyl phthalate/dioctyl phthalate oil (6:4, vol/vol), and cell-associated and free radioactivity were separated by microcentrifugation. The tubes were sectioned so as to separate the cell pellet and supernatant, and radioactivity was determined in a γ counter. Specific binding was determined as the difference between the amounts of ^{125}I bound to cells in the presence of medium (total binding) and in the presence of a 300-fold excess of unlabeled IL-1α (nonspecific binding). In this experiment, total binding was 7760 cpm, while the nonspecific binding was 860 cpm.

slightly different efficiencies. Irrelevant mAb had no inhibitory activity (data not shown). Each of the four hamster mAbs was more inhibitory than the M15 rat anti-type I IL-1 receptor mAb generated previously. In other experiments using radiolabeled ligand or radiolabeled antibody, IL-1α and JAMA-147 were found to bind to EL-4 with comparable affinities (2.3×10^9 and 1.3×10^9 M^{-1}, respectively), while M15 displayed a K_a 1/10th as high (2.1×10^8 M^{-1}) (data not shown). Table 1 demonstrates that the hamster mAbs were also highly effective in inhibiting IL-1-dependent T-cell pro-

Table 1. Inhibition of IL-1-dependent D10.G4 proliferation by JAMA mAbs

				Dose of mAb (μg/ml) to inhibit proliferation†	
Exp.	Antibody	Species	Specificity*	By 50%	By 100%
1	JAMA-83	Hamster	MuIL-1R_I	30.0	>100.0
	JAMA-84	Hamster	MuIL-1R_I	3.0	50.0
	JAMA-110	Hamster	MuIL-1R_I	1.0	50.0
	JAMA-147	Hamster	MuIL-1R_I	0.22	25.0
2	JAMA-147	Hamster	MuIL-1R_I	0.5	25.0
	B122	Hamster	MuIL-1β	10.0	100.0
	H22	Hamster	MuIFN-γ	>1000	>1000
	M15‡	Rat	MuIL-1R_I	>1000	>1000
	35F5§	Rat	MuIL-1R_I	25.0	>1000
	GR-20	Rat	MuIFN-γR	>1000	>1000

*Mu, murine; R_I, receptor type I; R, receptor.
†Background [^{3}H]thymidine incorporation was 4579 cpm (Exp. 1) and 3367 cpm (Exp. 2). Response of 2×10^4 D10.G4 cells to 50 pg of IL-1β + Con A was [^{3}H]thymidine incorporation of 150,000 cpm (Exp. 1) and 19,720 cpm (Exp. 2).
‡Immunex Corporation.
§Hoffmann–La Roche.

liferation, as determined by using the D10.G4 proliferation assay. Moreover, as demonstrated in experiment 2, JAMA-147 was a far more effective inhibitor than either of the two currently existing rat mAbs specific for the murine type I IL-1 receptor (M15 or 35F5). Competition binding analysis revealed that the four mAbs recognize three epitopes on the type I IL-1 receptor: JAMA-147 recognizes one epitope, JAMA-84 and JAMA-110 recognize a second, and JAMA-83 recognizes a third (data not shown).

Anti-IL-1 mAbs Inhibit the Elaboration of Activated Macrophages in *Listeria*-Infected Mice. When a pool of the three anti-IL-1 antibodies (anti-IL-1α, anti-IL-1β, and anti-type I IL-1 receptor) was used, a profound inhibition of the *in vivo* anti-*Listeria* response was observed in both C.B-17 and SCID mice (Fig. 3). The antibodies inhibited the elaboration of activated macrophages as determined by quantitating the number of PEM bearing I-A^d 3–4 days after infection. The expression of class II MHC molecules on peritoneal macrophages is known to be a faithful indicator of IFN-γ-dependent macrophage activation and has been extensively studied by us and others (2, 3, 20, 22, 23). Only 10–12% of the PEM from uninfected C.B-17 (Fig. 3*A*) or SCID (Fig. 3*B*) mice were I-A^d-positive, whereas 60–80% of the PEM from infected mice were class II MHC-positive. Irrelevant hamster mAb did not affect the induction of class II-positive cells. In contrast, PEM derived from infected animals treated with the anti-IL-1 mixture were only 9–10% class II-positive. Anti-IL-1 treatment of infected animals also resulted in systemic infection with increased mortality. In a representative experiment, the spleens of mice infected for 3 days with 5×10^3 *Listeria* and treated with either saline or irrelevant hamster mAbs contained 1.5×10^5 and 1.1×10^5 viable bacteria, respectively. Spleens from mice treated with the anti-IL-1 mixture contained 40–60 times more live organisms, 6.5×10^6. Moreover, although most of the infected control SCID mice did not die from the *Listeria* infection (survival = 13/14 saline treatment, 18/18 control antibody treatment), a significant percentage (7/14) of the anti-IL-1-treated mice died from the infection. In addition, anti-IL-1-treated infected mice showed bacterial abscesses with neutrophils appearing in the liver, spleen, and lung, while infected control mice did not. The reduced induction of activated macrophages caused by the anti-IL-1 mixture was comparable to or greater than the inhibition seen when animals were treated with anti-TNF (data not shown). Differences in the PEM populations derived from antibody-treated and control mice were also observed when the cells were examined morphologically. Macrophages from infected control mice were well spread, with abundant membrane ruffling and visible phase-lucent vesicles. In contrast, macrophages from mice treated with the mixture of IL-1 antibodies were round or oval and showed limited membrane activity.

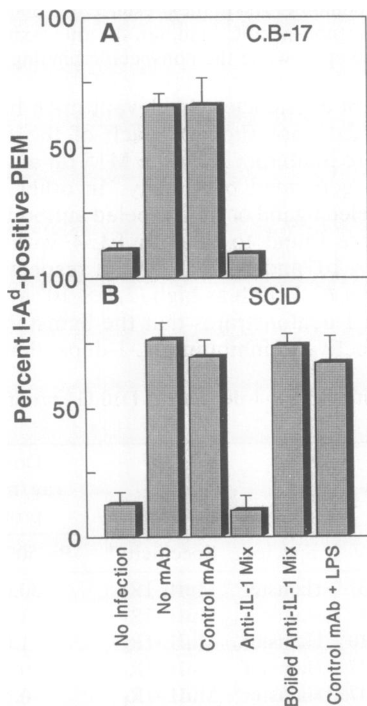

FIG. 3. Anti-IL-1 mAbs inhibit the induction of I-A^d on PEM derived from *Listeria*-infected normal (*A*) and SCID (*B*) mice. Mice were pretreated i.p. with saline (no infection, no mAb) or 600 μg of either purified irrelevant mAb (Control mAb L2-3D9) or a mixture (Anti-IL-1 Mix) containing 200 μg each of anti-IL-1α (ALF-161), anti-IL-1β (B122), and anti-type I IL-1 receptor (JAMA-147) on day −2 and then infected i.p. with 5×10^3 live *Listeria*. Additional controls received boiled anti-IL-1 mix or control mAb + lipopolysaccharide. Three days later mice were sacrificed, and PEM from individual mice were plated on coverslips, fixed in paraformaldehyde, and stained for I-A^d expression with the MKD6 mAb. Bars represent the mean + SD.

The effect of the anti-IL-1 mAb pool was specific and not due to contaminating endotoxin or IgG aggregates since (*i*) a control irrelevant hamster mAb prepared in the same manner was without effect (70% I-A^d-positive macrophages), (*ii*) the anti-IL-1 mAb preparation lost inhibitory activity when boiled (74% I-A^d-positive macrophages), and (*iii*) no inhibition was noted when the control mAb (600 μg per mouse) was spiked with *Escherichia coli* strain O111:B4 lipopolysaccharide (50 ng per mouse) (67% I-A^d-positive macrophages) (Fig. 3*B*).

The pool of mAbs was more effective in inhibiting the development of class II MHC-positive macrophages than any of the individual components (Fig. 4). However, the most effective component of the antibody mix was the anti-IL-1 receptor reagent.

The results presented herein, obtained with normal and SCID mice, clearly indicate that endogenously produced IL-1 has a profound effect *in vivo* on the processes that lead to

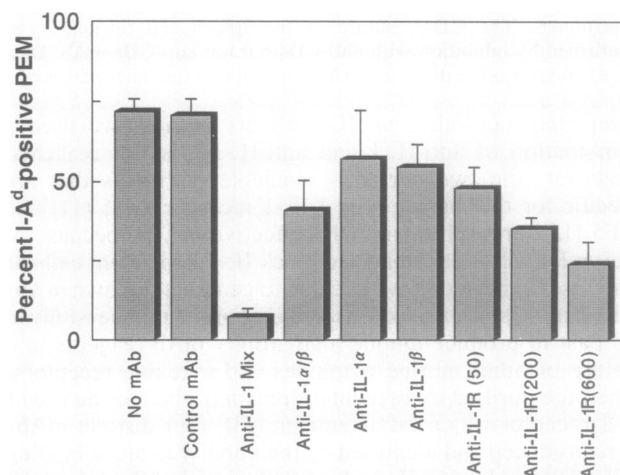

FIG. 4. Comparison of the ability of anti-IL-1α (ALF-161), anti-IL-1β (B122), and anti-IL-1 receptor (JAMA-147) mAb to inhibit class II MHC induction in *Listeria*-infected SCID mice. Mice were pretreated on day −2 with, from left to right, saline (n = 7), 200 μg of irrelevant L2-3D9 mAb (n = 7), the anti-IL-1 mixture containing 200 μg each of ALF-161, B122, and JAMA-147 (n = 8), a mixture containing 200 μg each of ALF-161 and B122 (n = 8), 200 μg of ALF-161 alone (n = 4), 200 μg of B122 alone (n = 4), or 50 (n = 4), 200 (n = 4), or 600 (n = 4) μg of JAMA-147 alone; subsequently the mice were infected on day 0 with 5×10^3 live *Listeria*. Class II MHC expression on PEM from individual mice was determined 3 days after *Listeria* injection. Bars represent the mean + SD.

macrophage activation and the eventual activation of anti-*Listeria* responses. However, on the basis of the data reported here and elsewhere, we do not believe that IL-1 directly effects the activation of macrophages, T cells, or NK cells. It is known that IL-1 does not directly induce expression of class II MHC molecules on macrophages (22). The inhibitory activity of the IL-1-specific antibodies in the SCID mouse and the lack of inhibition that the anti-IL-1 reagents had on the $CD4^+$ T-cell response to *Listeria* in the normal mouse rules out the possibility that the T cell was the target of IL-1 action. Finally, IL-1-specific antibodies do not inhibit the release of IFN-γ by SCID NK cells *in vitro* (2, 3). Therefore, the IL-1 effects must be ascribed to other steps in the inflammatory response. It is of interest that in preliminary experiments we have observed that polymorphonuclear leukocyte infiltrates at 24 hr were significantly reduced (to approximately 1/30th) in SCID mice treated with the anti-IL-1 mixture and then infected with *Listeria* compared with control infected animals treated with saline, irrelevant hamster mAb, anti-IFN-γ mAb (H22), or anti-TNF mAb (TN3-19.12). Thus, one role for IL-1 in this process may be to induce a local vascular response that attracts inflammatory cells (24) and/or to promote the release of proinflammatory cytokines, such as IL-8, that attract neutrophils or other cells (25). However, it is also possible that IL-1 mediates at least some of its effects by inducing the release of prostaglandins from macrophages [indomethacin profoundly inhibited resistance to *Listeria* (26)] or by recruiting NK cells to the site of infection (27).

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